

## **Dehydration and rehydration in *Selaginella sellowii* Hieron. aerial parts, a desiccation tolerant species**

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**RESUMO: (Desidratação e reidratação nas partes aéreas de *Selaginella sellowii* Hieron., uma espécie tolerante à dessecação)** *Selaginella sellowii* Hieron. é uma licófito tolerante à dessecação que cresce em afloramentos rochosos da região Neotropical. O objetivo deste estudo foi o de analisar o conteúdo relativo de água (CRA), carboidratos solúveis em água (CSA) e o conteúdo de clorofilas durante o progresso da dessecação e da reidratação. Plantas de *S. sellowii* foram coletadas com substrato e irrigadas à capacidade de campo. A seca foi induzida pela interrupção da irrigação. As amostragens da desidratação ocorreram a 0, 24, 48, 96 e 192 h de restrição hídrica. Para avaliar o efeito da reidratação nos parâmetros analisados, as plantas foram irrigadas e as amostras foram coletadas após 1, 2, 4, 10 e 24 h. Em restrição de água, as partes aéreas gradualmente perderam água em 48 h e exponencialmente até 96h, alcançando 3% de CRA em 192 h. O conteúdo de clorofilas decresceu até 96 h de dessecação. Os teores de CSA aumentaram em 24 h sem irrigação, seguido por um decréscimo gradual até 96 h. Quando o suprimento de água foi restabelecido, as partes aéreas apresentaram 85% de CRA 1 h. Após 10 h de reidratação, os conteúdos de clorofila e CSA chegaram aos níveis do início do experimento, o que indica a retomada do metabolismo. *S. sellowii* é uma espécie poiquilohídrica, homoeclorofílica e capaz de modular o metabolismo de CSA durante a dessecação e reidratação.

**Palavras-chave:** Carboidratos solúveis, Conteúdo de clorofila, Licófito, Planta revivescente, Poiquilohídrico.

**ABSTRACT:** *Selaginella sellowii* Hieron. is a desiccation-tolerant lycophyte which grows in rocky outcrops from the Neotropics. The aim of this study was

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to analyze the relative water content (RWC), water soluble carbohydrates (WSC) and chlorophyll contents during the progression of desiccation and rehydration. Plants of *S. sellowii* were harvested and irrigated to the field capacity. Drought was induced withholding irrigation. Dehydration sampling occurred at 0; 24; 48; 96 and 192 h of water shortage. In order to evaluate the rehydration effect in the analyzed parameters, plants were irrigated and sampled after 1; 2; 4; 10 and 24 h. Aerial parts lost water gradually in the first 48 h, and exponentially until 96 h. RWC reached 3% in 192 h. Chlorophyll content decreased until 96 h of desiccation. WSC levels increased after 24 h without irrigation, followed by a gradual decrease until 96 h. When water supply was reestablished, aerial parts reached 85% of RWC within 1 h. After 10 h of the rehydration, chlorophyll and WSC contents reached the initial levels, indicating the resumption of the metabolism. *S. sellowii* is a poikilohydric and homoiochlorophyllous species and can modulate WSC metabolism during desiccation and rehydration.

**Key words:** Water soluble carbohydrates, Chlorophyll content, Lycophyte, Resurrection plant, Poikilohydric.

## Introduction

Desiccation tolerance is the recognized ability of an organism to equilibrate its water potential with that of the environment and resume normal function after rehydration (Rascio & La Rocca, 2005). However, this feature is not widespread in vegetative organs of vascular plants. Desiccation tolerance has been demonstrated in about 350 species of vascular plants, including ferns and their allies, some monocotyledons and eudicots (Porembski & Barthlott, 2000). In desiccation-tolerant vascular plants, dehydration occurs relatively slowly, due to a combination of morphological and physiological mechanisms that delay the rate of water loss, providing to these plants appropriate conditions to set up the tolerance (Toldi *et al.*, 2009).

The main strategies of desiccation-tolerant plants to survive desiccation are related to deal with membrane and protein damage, mechanical and oxidative stresses (Rascio & La Rocca, 2005). In moderate drought, plants synthesize osmolytes, like water soluble carbohydrates, quaternary ammonium compounds and late embryogenesis abundant proteins, which may increase the water-holding capacity of cells, and also act in membrane and macromolecule protection (Hare *et al.*, 1998). Sucrose and trehalose are the main sugars proposed to contribute during drought stress, due to their ability promoting cytoplasm vitrification (Farrant & Moore, 2011). In the vitrified state, the

protection mechanism involves the physical limitation of the structures and the slowdown of chemical reactions. Thus, diffusion in the cytoplasm and free radicals production are limited (Hoekstra, 2005).

Another strategy involves chlorophyll content. Desiccation-tolerant plants that retain the chlorophyll content during desiccation are named homoiochlorophyllous. The species that lose almost entirely their chlorophyll and re-synthesize with rehydration are the poikilochlorophyllous (Oliver *et al.*, 2000). Both strategies may protect cells against oxidative damage due overreduction of the photosynthetic electron transport chain (Christ *et al.*, 2014). The homoiochlorophyllous limit the formation of reactive oxygen species (ROS) by leaf folding and curling, additionally by the overproduction of anthocyanins, but recover the photosynthetic activity promptly after rehydration. In another way, the poikilochlorophyllous simply remove the source of ROS formation but resynthesize the photosynthetic apparatus as soon as cells are rehydrated (Rascio & La Rocca, 2005).

Several desiccation tolerant species have been well studied. However, despite the abundance of desiccation-tolerant species in South America (Gaff, 1987), just a few studies focus these species.

*Selaginella sellowii* Hieron. is a hemicryptophyte distributed all over the Neotropics. This species is often found in soil islands with thin-layered substrate, on the inselbergs (Santos & Sylvestre, 2006). When hydrated, *S. sellowii* has approximately 10% of total sugars, consisting primarily of glucose and trehalose (Moraes *et al.*, 2014). Desiccation tolerance represents an advantage for plants during the occupation of land habitats (Bartels, 2005). Thus, the study of desiccation tolerance in vegetative organs of *S. sellowii* would contribute to the understanding of the plant adaptation mechanisms to water-limited niches.

In this study, the progression of dehydration and rehydration and its influence in chlorophyll and water soluble carbohydrate contents is showed in aerial parts of *S. sellowii* growing *ex situ*.

## Material and methods

Plants with substrate were collected in “Costão de Itacoatiara” rocky outcrop (22°58'33”- 22°59'00”S, 43°01'33”- 43°02'00”W), located in Niterói, RJ, Brazil, in April 2005. Voucher was deposited in the Herbarium of the Faculdade de Formação de Professores, Universidade do Estado do Rio de Janeiro (RFFP 6916). Squares with plants growing in substrate, with approximately 100 cm<sup>2</sup> were placed at the greenhouse being daily

irrigated to the field capacity during one week for acclimation. After that, the irrigation was suspended and sampling occurred at 24; 48; 96 and 192 h. Control plants were irrigated daily to the field capacity. Rehydration dynamics was verified when irrigation was suspended for 192 h in one group of plants. Following, plants were irrigated to the field capacity and sampled at 1; 2; 4; 10 and 24 h after rehydration. The reference time for sampling was at 8:00 am. In the experimental period, temperature varied from 25.2 to 31.5°C and air relative humidity at mid-day from 62.5 to 77%. We used four squares for each treatment.

Water content in the substrate was determined after drying approximately 1 g of substrate at 80 °C for 48 h. Water content was expressed as a percentage of substrate fresh mass (n = 4). Relative water content (RWC) in aerial parts was determined gravimetrically (Barr & Weatherley, 1962).

Pigments were extracted by the maceration of approximately 1.0 g of fresh material with 10 mL of cold aqueous acetone (80 % v/v) in the dark, followed by centrifugation at 2500 rpm for 10 min (n = 4). This procedure was repeated and the supernatants were pooled. Chlorophyll determination was made at 645 and 663 nm using the equations proposed by Arnon (1949). The results were expressed in mg.g<sup>-1</sup> DW.

For the water soluble carbohydrate (WSC) analyses, 2.0 g of fresh material of each sample (n = 4) were immediately placed in 10 mL of ethanol 80% at 80 °C for 5 min to enzyme inactivation. This procedure was repeated three times. Then the residues were extracted twice with distilled water at 60 °C for 30 min and filtered. Ethanolic and water supernatants were pooled and concentrated under vacuum (37 °C). WSC were determined by the anthrone method using glucose as standard (Yemm & Willis, 1954). All the spectrophotometric determinations were performed in triplicate for each sample.

Each treatment had four repetitions. Data were expressed as means ± standard deviation.

## Results

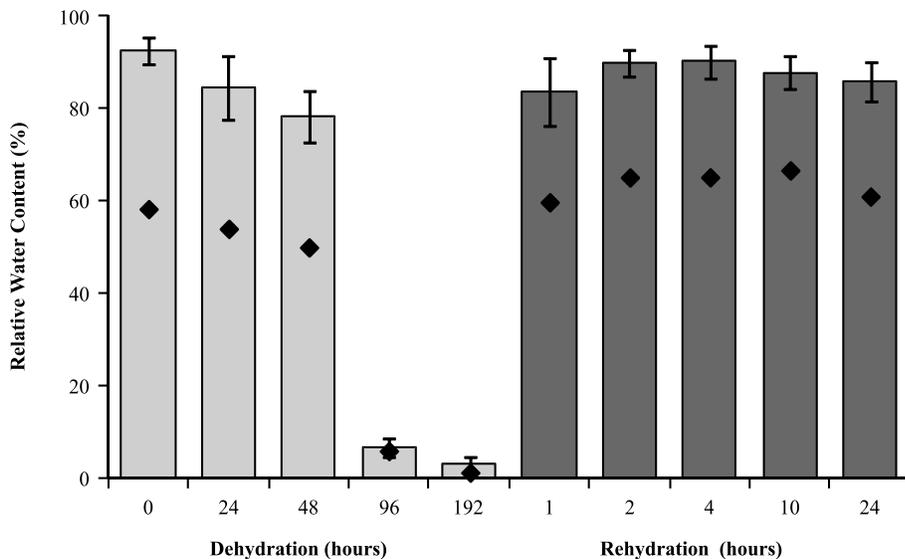
Water deprivation resulted in gradual wilting and curling of *S. sellowii* aerial parts in 8 days without irrigation. When well-irrigated (0 h), the aerial parts of the plants were fully expanded and green, covering most of the substrate. The relative water content was 92.2%. At 24 h plants remained expanded and green, with 84.4% of RWC. Following, at 48 h of water deprivation, RWC decreased to 78.2%, however without apparent curling of the aerial parts.

Desiccation was verified in 96 h and was characterized by wilting and curling of aerial parts, exposing the substrate, and by a drop in RWC to 6.8%. At 192 h plants remained curled and maintained the desiccated state, with 3.4% of RWC (Figure 1).

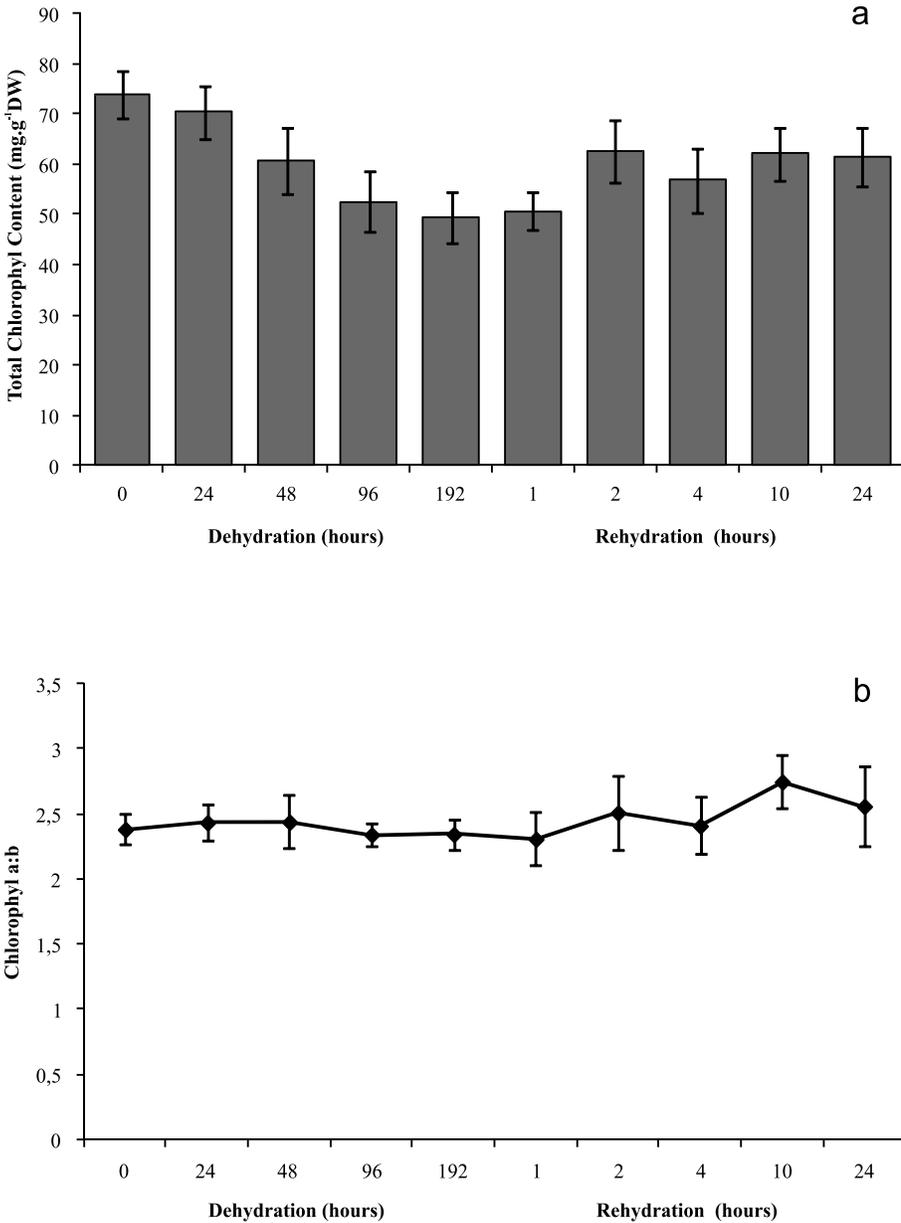
After irrigation, plants were quickly rehydrated, regained the turgor, expanded the aerial parts and reestablished the green color. This was observed one hour after irrigation, when RWC was 83.4%, and remained in this state until the 24 h evaluation (Figure 1).

A reduction of 30% in total chlorophyll content was observed after the 96 h in water deprivation, but chlorophyll levels remained constant in until 192 h of water deprivation. After rehydration, chlorophyll levels increased in 2 h, with a little oscillation in 4 h and remained constant until 24 h (Figure 2a). Chlorophyll a:b ratio was constant during dehydration and showed slight oscillation after rehydration (Figure 2b).

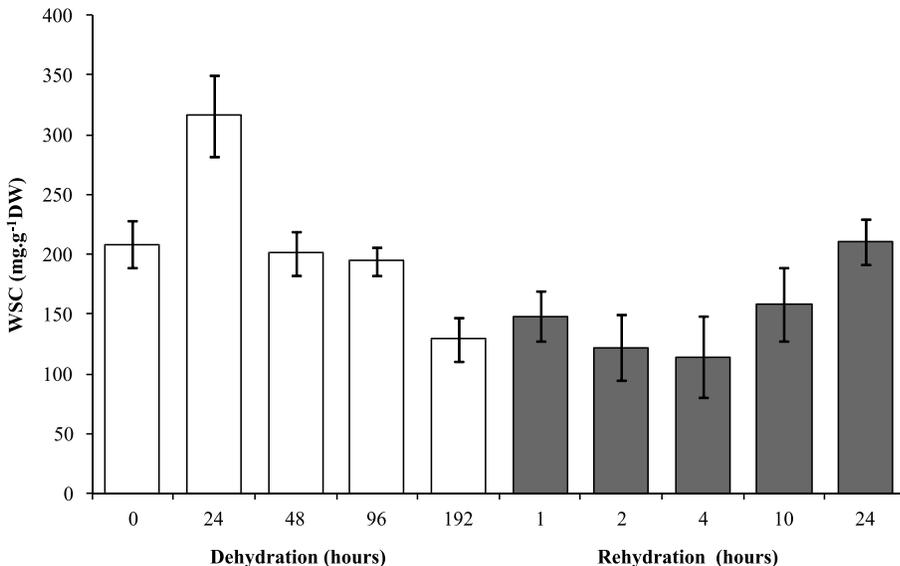
At the beginning of the treatment, the aerial parts of *S. sellowii* showed approximately 20% of WSC. This level increased in the first 24 h of dehydration, followed by a gradual decrease until the 192 h, when showed the lower WSC levels. After irrigation reestablishment, WSC levels increased after 10 h and reached the initial levels in 24 h after rehydration (Figure 3).



**Figure 1.** Relative water content of *Selaginella sellowii* plants growing *ex situ* submitted to dehydration followed by rehydration (n = 4). Lozenges indicate substrate water content (%). Columns are means and vertical bars the standard deviation.



**Figure 2.** Variation of total chlorophyll contents of aerial parts of *Selaginella sellowii* plants growing *ex situ* (n = 4), submitted to dehydration followed by rehydration (a) and ratio chlorophyll a:b (b). Columns are means and vertical bars the standard deviation.



**Figure 3.** Water soluble carbohydrate (WSC) content of aerial parts of *S. sellowii* plants growing *ex situ*, submitted to dehydration followed by rehydration (n = 4). Columns are means and vertical bars the standard deviation.

### Discussion

*S. sellowii* is a resurrection plant that can be characterized as poikilohydric. At the beginning of the experiment RWC was 92%, similar levels to the RWC of hydrated *S. sellowii* plants growing in rocky outcrops (Moraes *et al.*, 2014). In this study, we confirmed the extent of the dehydration tolerance in the aerial parts of *S. sellowii*, which in water deprivation desiccated and curled in 96 h. After 192 h of the irrigation suppression, RWC decreased to 3.4%. Desiccation-sensitive plants in active growth have RWC of 85 to 100% and die if RWC decreases from 59 to 30% (Gaff & Oliver, 2013). Air-dried resurrection plants usually have 4 to 13% RWC, and this value is used as a criterion for desiccation tolerance (Gaff, 1987; Toldi *et al.*, 2009). Some ferns and allies, including two *Selaginella* species, had 4.0 to 8.8% of RWC when desiccated (Gaff, 1987). Although there are *Selaginella* species which are mesophytic and desiccation-sensitive (Kessler & Siorak, 2007), some species of this genus are recognized as desiccation-tolerant, because they remain viable with low RWC levels, as exemplified by *S. lepidophylla* (Pampurova & Van Dijck, 2014), *S. tamariscina* (Liu *et al.*, 2008) and *S. bryopteris* (Pandey *et al.*, 2010).

Cells of desiccation-tolerant plants undergo to several changes during desiccation, since water is essential to maintain the configuration of membranes and proteins. In a substantial drying with RWC lower than 3%, strategies of desiccation tolerance are needed in order to maintain the physiological and structural integrity (Rascio & La Rocca, 2005). If desiccation is well succeeded, cells will be able to rehydrate and resume their functions as observed in *S. sellowii*.

Once irrigation was resumed, the leaves of *S. sellowii* quickly re-expanded and recovered hydration (RWC 85%), as verified one hour after irrigation (Figure 1). Also, chlorophyll (Figure 2) and WSC levels increased after rehydration (Figure 3). This attends the definition of desiccation tolerance that requires survival following lost the significant fraction of water of tissues exposed to dry conditions, and the recovering of full physiological competence after rehydration (Lüttge *et al.*, 2011). However, desiccation-tolerant plants show differences in rehydration, which is dependent on plant morphology and the extent of subcellular repair and reconstitution required for the resumption of metabolism (Sherwin & Farrant, 1996). The fast recovery in RWC observed in desiccated *S. sellowii* plants may facilitate the reestablishment of cellular functions.

*S. sellowii* can be classified as homoiochlorophyllous because chlorophyll content was retained during desiccation, even with a slight loss, and recovered in 24 h after rehydration (Figure 2a). Decreases in chlorophyll content to 50% during desiccation can occur in homoiochlorophyllous species, however succeeding 24 to 45 h of the rehydration, chlorophyll levels are recovered (Kappen & Valladares, 2007). This may be due to leaf curling that avoids light-chlorophyll interactions and results in chlorophyll shading and masking (Sherwin & Farrant, 1996). Frond curling has also been noted in *Selaginella lepidophylla* (Pampurova & Van Dijck, 2014) and in other desiccation-tolerant species. This is typical of homoiochlorophyllous angiosperm resurrection plants (Farrant *et al.*, 2003) and seems to be a mechanism for avoiding photoinhibitory damage (Porembski, 2011). In homoiochlorophyllous species, even before chlorophyll contents reach pre-desiccation levels, photochemical activity can be resumed (Alpert, 2006). Thus, carbon assimilation may be recovered.

WSC metabolism oscillated with dehydration and rehydration. Increases in the levels of osmotically active compounds, like WSC, constitute a response to drought, even in desiccation-sensitive species (Ober *et al.*, 2005). In *S. sellowii*, the initial increase in WSC may contribute to RWC maintenance until 48 h of the irrigation suppression (Figure 1). After 10 h of rehydration, the rise in WSC levels indicates the resumption of carbon metabolism (Figure 3). WSC are recognized as protective molecules during stress by the combination

of properties. Additionally to the maintenance of osmotic pressure, sugars may protect membranes during stress (Hinch *et al.*, 2007) and form biological glasses (Crowe *et al.*, 1998). In *S. sellowii* the main WSC are glucose and trehalose (Moraes *et al.*, 2014). This disaccharide has protective roles during stress and is often accumulated in poikilohydric plants (Farrant & Moore, 2011; Gaff & Oliver, 2013). In *Selaginella*, trehalose is one of the major WSC components. Recently trehalose was reported in desiccation-tolerant and desiccation-sensitive species, and its role as the responsible for desiccation tolerance has been argued (Pampurova & Van Dijck, 2014).

Desiccation tolerance in vascular plants is a very complex subject, which depends on the interaction of several factors (Rascio & La Rocca, 2005). In *S. sellowii*, some of the basic mechanisms were characterized, but how long these plants will persist alive under desiccation, other physiological, biochemical and molecular mechanisms in dehydrated and rehydrated plants remain to be cleared.

### Conclusion

*S. sellowii* is a poikilohydrous and homoiochlorophyllous species and can modulate WSC metabolism during desiccation and rehydration.

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